FLUXION

APPLICATION NOTE

B'SYS CHO hERG+ Characterizeation and Functional Validation with IonFlux Single Mode HT.

Introduction

B'SYS CHO hERG+ cells from B'SYS GmbH were characterized at Fluxion Biosciences and validated using IonFlux HT single cell F1 plates.

This report contains the characterization of the B'SYS CHO hERG cells in regard to:

- Longevity of Current (Run-down)
- Current Success
- Gigaseal Success
- Biophysical Characterization
- Pharmacology Characterization

Methods

Culturing:

B'SYS CHO (Chinese Hamster Ovary) hERG+ human ERG (ether-a-go-go related gene) cells were cultured following B'SYS guidelines (B'SYS GmbH, Switzerland, info@bsys.ch).

In brief, B'SYS CHO hERG Complete Media contained F-12 (HAM) with GlutaMAX (31765-Invitrogen), 10% FBS (F2442 - Sigma), 1.0% Penicilin/Strepomycin (15140-Invitrogen), 100Mg/ml Hygromycin (10689 - Invitrogen), and 100Mg/ml Neomycin (G418; 10131 - Invitrogen).

The cells were incubated in a 30°C incubator for 1-5 days before running experiments to boost hERG-CHO expression. Cells were detached using Detachin (100077-508 VWR) for 15 minutes at 37°C.

Solutions:

Extracellular Ringer's Solution
2 CaC_{l2}, 1 MgC_{l2}, 10 HEPES, 4 KCl, 145 NaCl, *10 Glucose (in mM)
pH = 7.4 (with NaOH) 305 mOSM (with sucrose)

Intracellular Ringer's Solution
5.374 CaC_{l2}, 1.75 MgC_{l2}, 10 EGTA, 10 HEPES, 120 KCl, *4 N_{a2}-ATP (in mM)
pH = 7.2 (with KOH) 295 mOsm (with sucrose)

Extracellular Ringer's was approximately 10 mOsm higher.

Verapamil was purchased from Sigma. Verapamil was first dissolved in DMSO as a high concentration stock solution (10 mM), then diluted in dose series in DMSO and FL Reagent (Fluxion Biosciences) before being diluted into the final concentrations in the ECS. The final concentration of DMSO was equal in the same dose series (0.1%). A negative control of DMSO solution (0.1%) was always applied before compound applications, and was not found to induce a change in current amplitudes exceeding 10%.

Protocols

A sample voltage protocol is shown below (figure 1) for evoking hERG current. Every 15 seconds there was a 5 second depolarizing voltage step (from -80 mV to +20mV) which was followed by a 5 second tail step to -50mV. Example raw currents for one zone (16 traps) are shown below the voltage protocol.

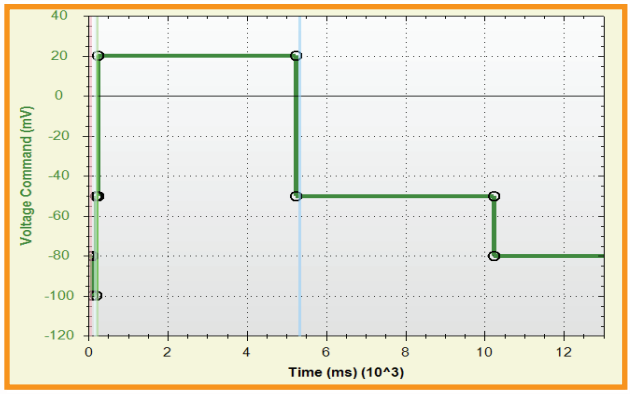
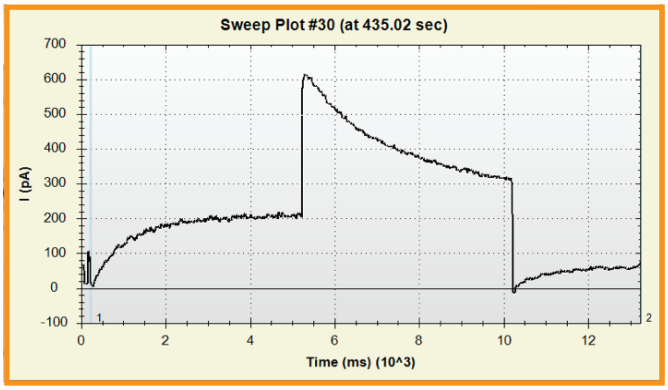


Figure 1. Example of raw hERG current sweeps are shown below the voltage protocol



Success Rate in Single Cell Mode and Stability of Recordings

A sample hERG current trace is shown below in figure 2 for one zone (16 traps) on an IonFlux single cell F1 HT plate. In this example, there were (~69%) successful recordings showing expression levels ranging from at least 200 pA in peak tail current measurements.

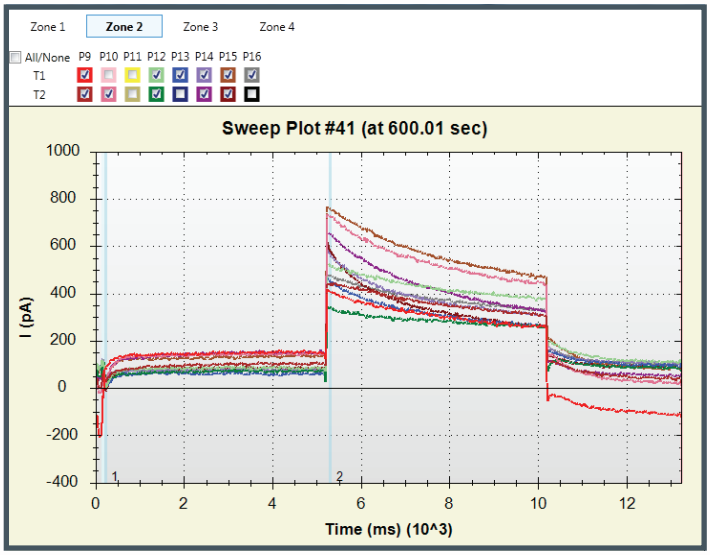


Figure 2. Example of raw superimposed hERG current sweeps are shown for one zone.

B'SYS CHO hERG+ cells showed stability in current measurements on the IonFlux even up to ~40 minutes of recording. A sample hERG current versus time trace is shown in figure 3. In this trace, current expression ranged from ~100-900 pA with a success rate of ~81%. The majority of currents were stable during the ~40 minutes of recording with minimal run-down observed.

The graph below (figure 4) shows a summary of B'SYS CHO hERG+ current percentage success for 5, 10, 15, and 20 minute time points (average +/- SEM). The success rates using a 200 pA threshold were above 50% for all time points tested (N=5 plates).

Resistance percentage success were measured at 5, 10, 15 and 20 minute time points (see Figure 5 below, (Average +/- SEM). Gigaseal success (with an 800 M Ohm Threshold) were above 50% (N= 5 plates) for all of these time points.

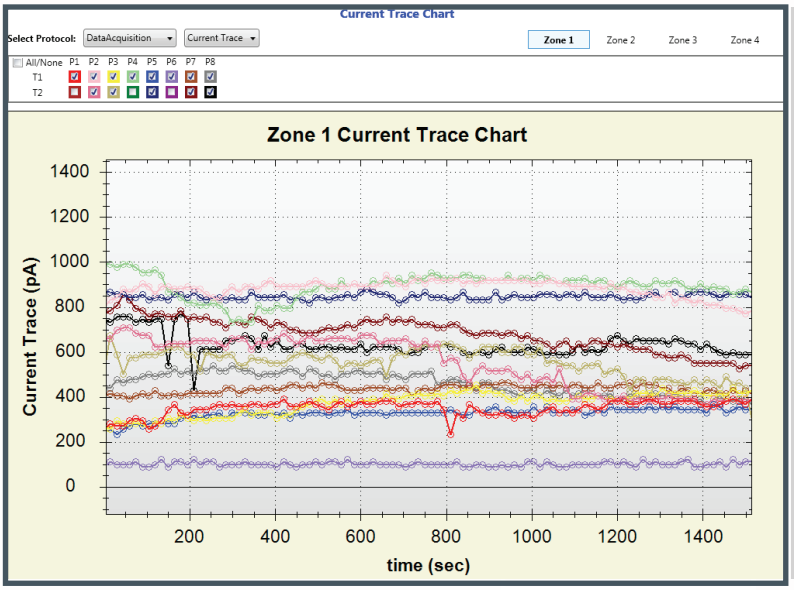


Figure 3. Example of hERG current versus time trace is shown for one zone

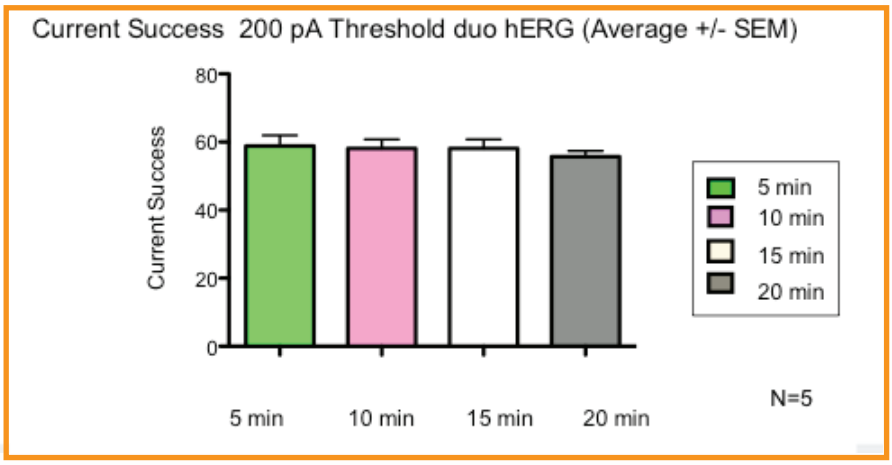


Figure 4. Percentage of hERG current success for 5, 10, 15, and 20 minute time points

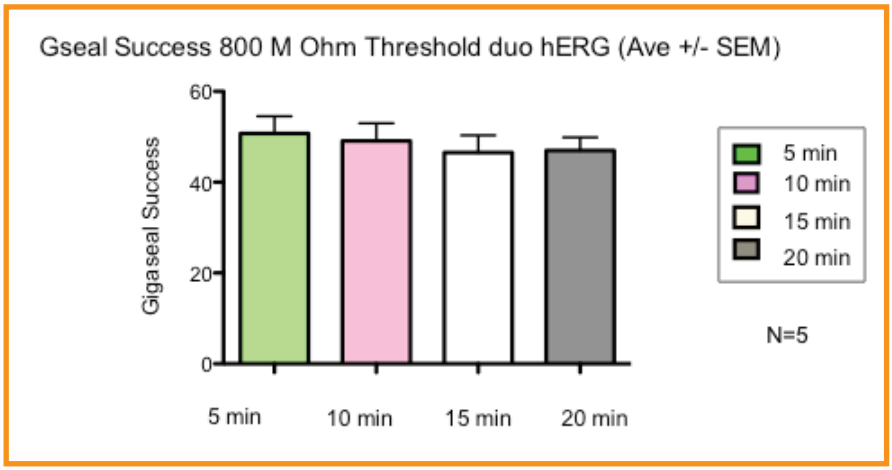


Figure 5. Percentage of hERG Gigaseal current success for 5, 10, 15, and 20 minute time points

In addition, the average resistance values for these same time points are shown in figure 6. The average resistance values were above 1 GigaOhm for all time points.

hERG Biophysical Characterization

hERG currents were evoked by the following voltage protocol - every 15 seconds, there was a 5 second depolarizing voltage step (from -80mV to +50 mV) followed by a 5 second tail current. A raw current is shown in figure 7.

The hERG steady rate current increased in ampitude until 0mV. At more positive values, the hERG current decreased due to fast inactivation. The I/V relationship was bell shaped (figure 8).

The hERG tail current was stepped from -120mV to -10mV (in 10mV intervals from a holding potential of -80mV. At potentials that were negative to the reversal potential (~-77mV) inward tail currents were obseved (figures 9 & 10).

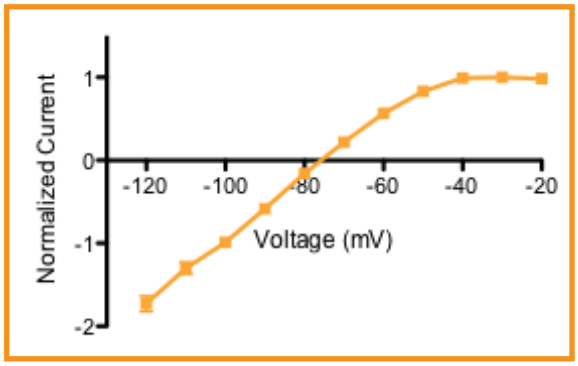


Figure 10. Normalized tail current plotted against voltage. Erev = 77 mV (mean +/- SEM , n=18)

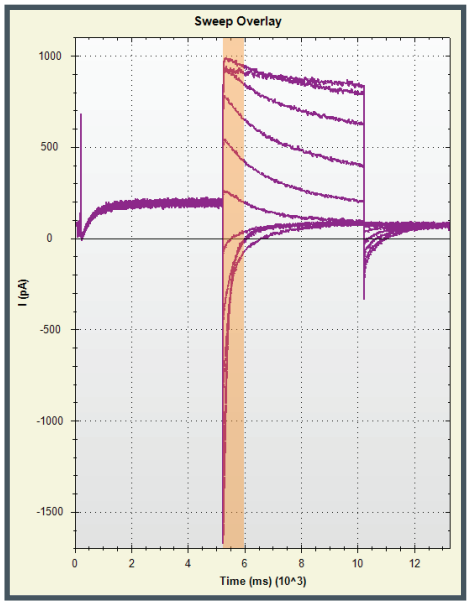


Figure 9. Raw tail current data.

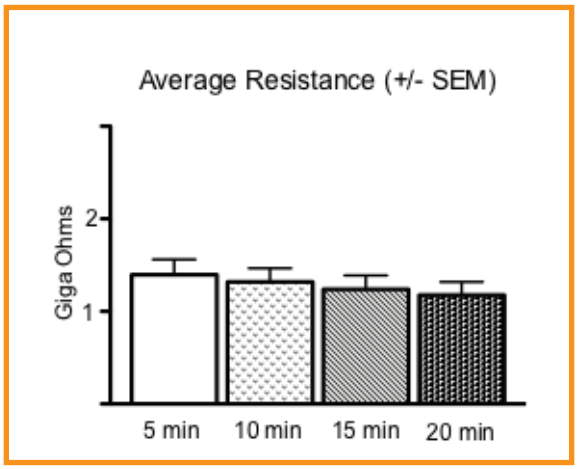


Figure 6. Average resistance values for 5, 10, 15, and 20 minute time points.

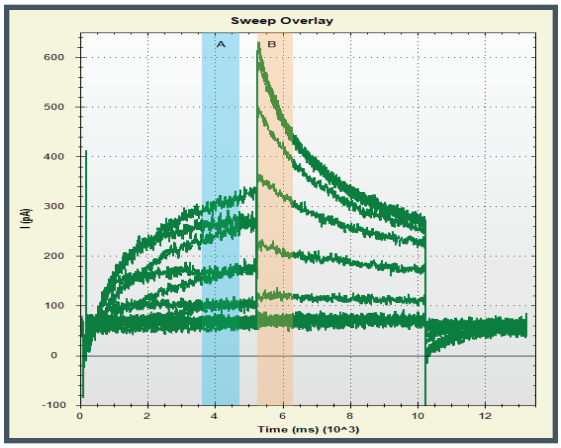


Figure 7. Raw current traces for tail current biophysical characterization. Cursor "A" was used for hERG I/V current characterization whereas cursor "B" was used for tail current characterization

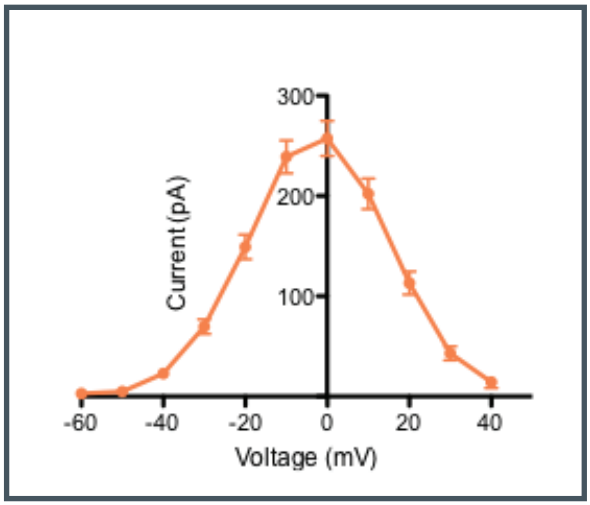


Figure 8. I/V relationship for hERG (mean +/- SEM).

Pharmacology

Verapamil was used to characterize the B'SYS CHO hERG+ pharmacology. The voltage protocol in figure 1 was used for this pharmalogical characterization. Verapamil was applied in four increasing concentrations with a 10 fold dilution starting from 0.001 Mm. The compound plate setup is shown below in figure 11.

Pattern Compound	Well	Cell ID	Compound A	Concentration A
P1 - C1	A3	BSYS hERG CHO +	Saline	0
P1 - C2	A4	BSYS hERG CHO +	Verapamil	0.001
P1 - C3	A5	BSYS hERG CHO +	Verapamil	0.01
P1 - C4	A6	BSYS hERG CHO +	Verapamil	0.1
P1 - C5	B6	BSYS hERG CHO +	Verapamil	1
P1 - C6	B5	BSYS hERG CHO +	Verapamil	10

Figure 11. Sample plate compound information for a 4 point dose response Verapamil experiment.

Example raw current traces from the IonFlux Sweep Overlay window are shown below in figure 12. One trace is shown per application of Verapamil starting with the control (saline) trace. Figure 13 shows an example current versus time plot showing the effect of increasing applications of Verapamil on hERG current. The steady state values at the end of each application were used for Hill plots.

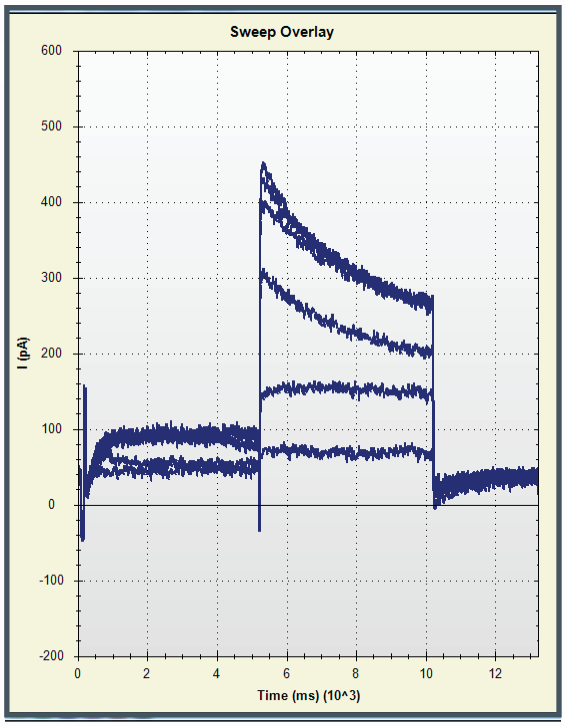


Figure 12. hERG current raw traces. One trace is shown per application of Verapamil (starting with a control saline trace).

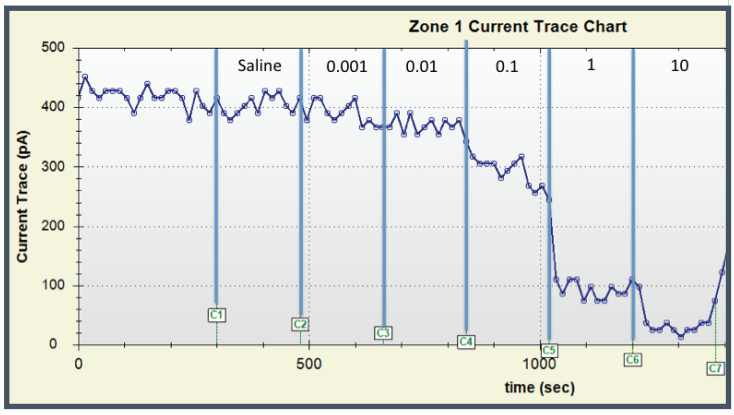


Figure 13. Current versus time plot showing the effect of increasing applications of Verapamil on an example current.

The Hill fit using the average blocker effents in a verapamil 5 point dose response experiment showed an IC50 = 190 nM (N=16). The graph for this fit is shown in Figure 14.

Conclusion

The B'SYS CHO hERG+ cell line was characterized with IonFlux HT single cell F1 plates. The cells showed stable currents with current success above 50% . Run down was minimal for hERG currents. Pharmacology of the hERG current was tested with Verapamil with five increasing concentrations of Verapamil. The 5 dose response experiment for Verapamil showed an IC50 = 190 nM (N=16).

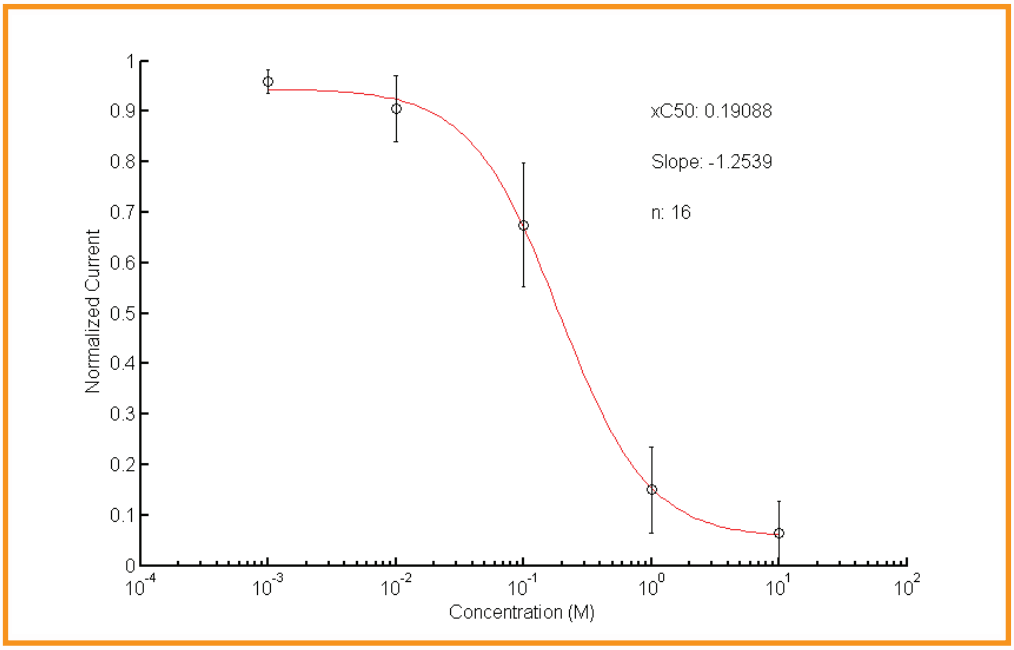


Figure 14. Hill plot for the average current in a 5 dose response Verapamil experiment. IC50=190nM (N=16)